

**You might find this additional information useful...**

This article cites 112 articles, 88 of which you can access free at:

<http://ajprenal.physiology.org/cgi/content/full/277/5/F665#BIBL>

This article has been cited by 13 other HighWire hosted articles, the first 5 are:

**Parathyroid hormone stimulates the endothelial nitric oxide synthase through protein kinase A and C pathways**

G. Rashid, J. Bernheim, J. Green and S. Benchetrit

*Nephrol. Dial. Transplant.*, October 1, 2007; 22 (10): 2831-2837.

[Abstract] [Full Text] [PDF]

**Defective coupling of apical PTH receptors to phospholipase C prevents internalization of the Na<sup>+</sup>-phosphate cotransporter NaPi-IIa in Nherf1-deficient mice**

P. Capuano, D. Bacic, M. Roos, S. M. Gisler, G. Stange, J. Biber, B. Kaissling, E. J. Weinman, S. Shenolikar, C. A. Wagner and H. Murer

*Am J Physiol Cell Physiol*, February 1, 2007; 292 (2): C927-C934.

[Abstract] [Full Text] [PDF]

**Parathyroid hormone-related protein regulates intestinal calcium transport in sea bream (*Sparus auratus*)**

J. Fuentes, J. Figueiredo, D. M. Power and A. V. M. Canario

*Am J Physiol Regulatory Integrative Comp Physiol*, November 1, 2006; 291 (5): R1499-R1506.

[Abstract] [Full Text] [PDF]

**Osteogenic regulation of vascular calcification: an early perspective**

R. Vattikuti and D. A. Towler

*Am J Physiol Endocrinol Metab*, May 1, 2004; 286 (5): E686-E696.

[Abstract] [Full Text] [PDF]

**Purification and Characterization of a Receptor for Human Parathyroid Hormone and Parathyroid Hormone-related Peptide**

M. Shimada, X. Chen, T. Cvrk, H. Hilfiker, M. Parfenova and G. V. Segre

*J. Biol. Chem.*, August 23, 2002; 277 (35): 31774-31780.

[Abstract] [Full Text] [PDF]

Medline items on this article's topics can be found at <http://highwire.stanford.edu/lists/artbytopic.dtl> on the following topics:

Oncology .. Parathyroid Hormone Receptors

Chemistry .. Peptide Ligand

Oncology .. Parathyroid Hormones

Physiology .. Bone Formation

Physiology .. Homeostasis

Medicine .. Osteoporosis

Updated information and services including high-resolution figures, can be found at:

<http://ajprenal.physiology.org/cgi/content/full/277/5/F665>

Additional material and information about *AJP - Renal Physiology* can be found at:

<http://www.the-aps.org/publications/ajprenal>

This information is current as of January 3, 2008 .

## Receptors for PTH and PTHrP: their biological importance and functional properties

MICHAEL MANNSTADT,<sup>1</sup> HARALD JÜPPNER,<sup>1,2</sup> AND THOMAS J. GARDELLA<sup>1</sup>

<sup>1</sup>Endocrine Unit, Department of Medicine and <sup>2</sup>Pediatrics, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

**Mannstadt, Michael, Harald Jüppner, and Thomas J. Gardella.**

Receptors for PTH and PTHrP: their biological importance and functional properties. *Am. J. Physiol.* 277 (Renal Physiol. 46): F665–F675, 1999.—The type 1 receptor (PTH1R) for parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrP) is a G protein-coupled receptor that is highly expressed in bone and kidney and mediates in these tissues the PTH-dependent regulation of mineral ion homeostasis. The PTH1R also mediates the paracrine actions of PTHrP, which play a particularly vital role in the process of endochondral bone formation. These important functions, the likely involvement of the PTH1R in certain genetic diseases affecting skeletal development and calcium homeostasis, and the potential utility of PTH in treating osteoporosis have been the driving force behind intense investigations of both the receptor and its peptide ligands. Recent lines of work have led to the identification of constitutively active PTH1Rs in patients with Jansen's metaphyseal chondrodysplasia, the demonstration of inverse agonism by certain ligand analogs, and the discovery of the PTH-2 receptor subtype that responds to PTH but not PTHrP. As reviewed herein, a detailed exploration of the receptor-ligand interaction process is currently being pursued through the use of site-directed mutagenesis and photoaffinity cross-linking methods; ultimately, such work could enable the development of novel PTH receptor ligands that have therapeutic value in treating diseases such as osteoporosis and certain forms of hypercalcemia.

parathyroid hormone; peptide hormone family; class II G protein-coupled receptor; receptor binding; signal transduction; structure-activity relationship; receptor mutagenesis; photochemical cross-linking; constitutively active receptor; receptor mutations in human disease; parathyroid hormone-related peptide

IN MAMMALS, parathyroid hormone (PTH) is the most important regulator of calcium ion homeostasis (62, 84). The peptide hormone is synthesized as a precursor protein containing a presequence of 25 amino acids and a prosequence of 6 amino acids, which are both cleaved during the synthesis and secretion process to yield the mature form of 84 amino acids. PTH is almost exclusively produced by the parathyroid glands (small amounts of its mRNA have been detected in the rat hypothalamus, Ref. 79), and its synthesis and secretion is largely regulated by the extracellular concentration of calcium, which is monitored by the calcium-sensing receptor of the parathyroid glands (16). In response to low blood calcium levels, PTH is secreted into the circulation and then acts primarily on kidney and bone, where it binds to cells expressing the type 1 PTH/PTHrP receptor (PTH1R). The ensuing direct and indirect responses of these target cells help to maintain blood calcium concentrations to within narrow limits.

In kidney, PTH directly stimulates the tubular reabsorption of calcium, and it stimulates the activity of  $1\alpha$ -hydroxylase and thereby increases the 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>]-dependent absorption of calcium from the intestine. In bone, PTH can induce a rapid release of calcium from the matrix, but it also mediates longer-term changes in calcium metabolism by acting directly on osteoblasts and indirectly on osteoclasts, the bone-resorbing cells. PTH action on osteoblasts leads to changes in the synthesis and/or activity of several proteins, including osteoclast-differentiating factor, also known as TRANCE, RANKL, or osteoprotegerin ligand (86, 122).

In addition to these regulatory actions on calcium homeostasis, PTH helps to maintain blood phosphate concentration within normal limits by inhibiting its reabsorption in proximal and distal tubules of the kidney (15, 18). This is achieved by reducing, through several different mechanisms, the expression levels of

the sodium-dependent cotransporter Npt2 (previously termed NaPi2) in the brush-border membrane of the proximal tubules. This reduction in Npt2 results in increased urinary losses of phosphate (70, 81). Although PTH exerts important effects on renal phosphate handling, it is likely that other factors, particularly dietary phosphate and a poorly characterized humoral factor provisionally termed "phosphatonin," are more important regulators of phosphate homeostasis (23).

#### PARATHYROID HORMONE-RELATED PEPTIDE

PTH-related peptide (PTHrP) was first discovered as the most frequent cause of the syndrome of humoral hypercalcemia of malignancy (71, 77, 97, 98, 101). However, PTHrP mRNA is widely expressed under normal conditions, and gene ablation experiments have established that this peptide plays an essential role in normal skeletal development (57). Human PTHrP can be produced as a 141-amino acid peptide or, through alternative mRNA splicing, as a protein comprising either 139 or 173 amino acids. PTHrP binds to the same receptor as PTH, and the biological responses elicited by either ligand through this common PTH1R are largely indistinguishable, at least with regard to mineral ion homeostasis (24, 26, 43, 59). For these actions of PTH and PTHrP, the amino-terminal (1–34) peptide fragments are sufficient, as PTH-(1–34) and PTHrP-(1–34) display high-affinity receptor binding and efficient receptor activation. There is a growing body of evidence, however, suggesting that midregional and/or carboxy-terminal fragments of either peptide, derived through posttranslational processing mechanisms, also have biological activity (61, 84, 119). However, the observed activities of midregional and COOH-terminal fragments of PTH and PTHrP are unlikely to be related to adult mineral ion homeostasis and are probably mediated through receptors that are distinct from the PTH1R, although these receptors have not yet been identified.

#### STRUCTURE-ACTIVITY RELATIONS IN PTH AND PTHRP

PTH and PTHrP show significant sequence homology within the first 13 amino acid residues (Fig. 1), and this sequence conservation reflects the functional importance of the amino-terminal residues in receptor signaling (38, 43, 44, 59, 107). Between PTH and PTHrP, sequence homology decreases markedly in the 14–34 region, where only three amino acids are identical, and beyond residue 34 there is no recognizable similarity. For both PTH and PTHrP, the 15–34 region functions as the principal PTH1R binding domain, and these portions of the two peptides probably interact with overlapping regions of the receptor, as the two fragments compete equally for binding with radiolabeled PTH-(1–34) or PTHrP-(1–36) to the PTH1R (1, 17). These data also suggest that the two divergent receptor-binding domains of PTH and PTHrP adopt similar conformations.

The three-dimensional crystal structures of PTH or PTHrP are not known, but the peptides have been

analyzed extensively by nuclear magnetic resonance (NMR) spectroscopic methods. In general, these studies indicate that, under certain solvent conditions, PTH-(1–34) and PTHrP-(1–36) analogs contain defined segments of secondary structure, including a relatively stable  $\alpha$ -helix in the carboxy-terminal receptor-binding domain, a shorter less stable helix near the amino-terminal activation domain, and a flexible hinge or bend region connecting the two domains (4, 73, 80). Although most NMR solution studies find evidence for peptide flexibility, the question of whether the conformations of PTH and PTHrP recognized by the receptor are folded with tertiary interactions, as suggested by some studies (5, 20, 37), or extended, as suggested by other analyses (80), remains unanswered.

#### RECEPTORS FOR PTH AND PTHRP

As indicated above, PTH and PTHrP mediate their actions primarily through the PTH1R PTH/PTHrP receptor, a G protein-coupled receptor (GPCR) with seven membrane-spanning helices. The PTH1R forms, along with the receptors for secretin, calcitonin, glucagon, and several other peptide hormones, a distinct family of GPCRs that exhibit none of the amino acid sequence motifs found in the other subgroups of the superfamily of heptahelical receptors (49, 60, 93). These peptide hormone receptors, called class II or family B receptors (60), can be distinguished from other GPCRs by their large (~150 amino acid) amino-terminal extracellular domain containing six conserved cysteine residues, as well as by several other conserved amino acids that are dispersed throughout the NH<sub>2</sub>-terminal domain, the membrane-embedded helices, and the connecting loops.

#### PTH1R SIGNALING AND LIGAND INTERACTIONS

The PTH1R is considered to be a potentially important target for pharmacological interventions aimed at treating disorders of mineral ion homeostasis and the skeleton, such as hyperparathyroidism, humoral hypercalcemia of malignancy, and osteoporosis. Studies on ligand interactions with the PTH1R have largely focused on amino-terminal peptide hormone analogs, such as PTH-(1–34) and PTHrP-(1–34), as there is no evidence to suggest that midregional or carboxy-terminal portions of the intact ligands interact with this receptor (48, 83, 102). Stimulation of cells expressing the PTH1R by PTH-(1–34) or PTHrP-(1–34) agonist ligands can activate at least two second messenger signaling systems: the adenylyl cyclase/protein kinase A (AC/PKA) pathway and the phospholipase C/protein kinase C (PLC/PKC) pathway (84, 92). The classic PTH1R-mediated cAMP/PKA pathway has been widely studied in a variety of cellular settings and typically elicits a robust and sensitive response to agonist ligands. In comparison, agonist efficacy and potency profiles observed in assays of the PLC/PKC pathway are generally lower than those of the AC/PKA pathway (39, 47).

The roles of these two signaling pathways in mediating the downstream physiological effects of PTH and

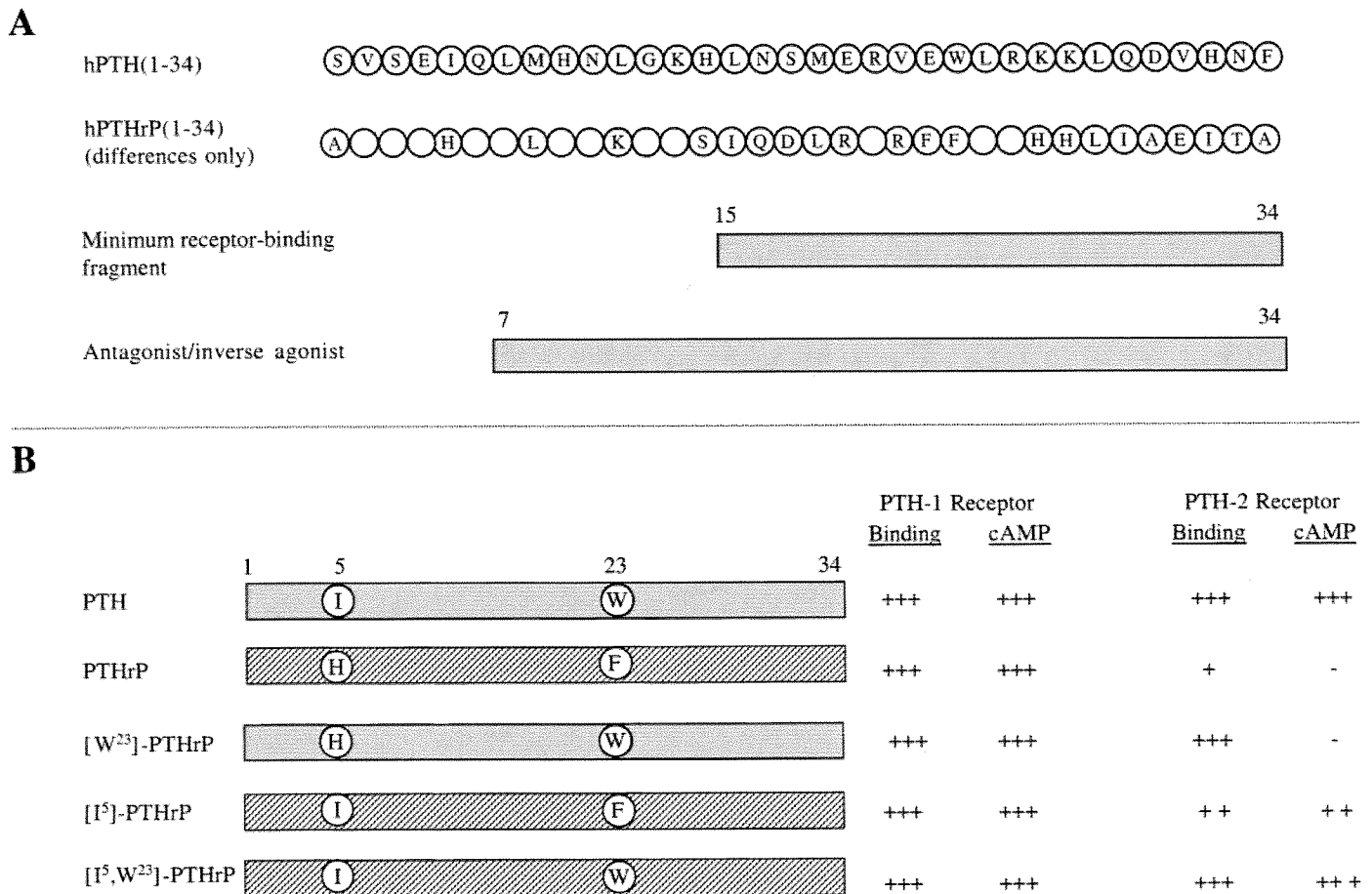


Fig. 1. Parathyroid hormone (PTH) and PTH-related peptide (PTHrP) and analogs: functional domains and receptor selectivity determinants. A: amino acid sequences of the bioactive (1–34) regions of native human PTH and human PTHrP. In the schematic of human PTHrP, only residues that differ from human PTH are provided, and amino acids that are identical to the corresponding residues of PTH are represented by open circles. Bars represent peptide fragments that exhibit weak receptor binding (15–34) or antagonist and inverse agonist properties (7–34), as described in the text. B: changing residues 5 and 23 in PTHrP to the corresponding residue of PTH rescues the inability of native PTHrP to bind to and stimulate cAMP formation in cells expressing the PTH-2 receptor. Note that the PTH-1 receptor does not discriminate between these analogs.

PTHrP are still poorly understood, but some progress has been made at deciphering the mechanisms by which the two signals are transduced at the membrane level. It has been well established that the determinants of cAMP/PKA signaling in the ligands PTH and PTHrP reside within the amino-terminal residues (38, 43, 59, 107); however, the ligand determinants of PLC/PKC activation have been more difficult to define. In some recent studies, a PTH-based tetrapeptide containing only residues 28–32 activated PKC activity in a rat osteosarcoma cell line (31, 51, 52); yet PTH analogs as short as 1–30 activated PLC in cells transfected with the recombinant rat or human PTH1R (103). Moreover, PTH-(3–34) was devoid of PLC-stimulating activity in similarly transfected cells (104). At present, the non-adenylyl-cyclase-mediated pathways appear more complex than the AC/PKA pathway and may involve multiple phospholipase isoforms (e.g., PLD and PLC), and be sensitive to variations in cell type, receptor density, and receptor species derivation (28, 103, 104).

The receptor sites involved in coupling to G proteins are currently being investigated by mutagenesis methods. For example, mutations in the second intracellular loop of the rat PTH1R selectively uncouple the G<sub>q</sub>-linked pathway (e.g., PLC-signaling is impaired) without disrupting the G<sub>s</sub>-linked activation of AC (46), and mutations in the third cytoplasmic loop uncouple both the G<sub>s</sub>- and the G<sub>q</sub>-linked pathway (45). Subsequent to G protein activation, the PTH1R becomes phosphorylated and desensitizes (12, 13, 69, 85), and an area of current investigation involves the use of novel receptor mutants and fluorescent microscopy techniques to define the biochemical linkages between these processes (105).

The mechanisms by which PTH and PTHrP bind to the PTH1R and then induce the presumed conformational changes that lead to G protein coupling have been explored through the use of receptor mutants and receptor chimeras. Structurally altered PTH or PTHrP analogs have proven to be of value in these studies by serving as functional probes of ligand interaction sites

in the receptor. For example, the antagonist PTH-(7-34) bound to the human PTH1R with ~30-fold higher affinity than it did to the rat PTH1R. Reciprocal pairs of rat/human and human/rat receptor chimeras were used to identify receptor regions involved in this binding selectivity, and the results identified the amino-terminal extracellular domain as the major determinant (55). Similarly, studies on the analog [Arg<sup>2</sup>]PTH-(1-34), which is an agonist with the opossum PTH1R and an antagonist with the rat PTH1R, revealed clues as to receptor residues involved in recognizing the side chain of position 2 in the ligand. Thus rat/opossum PTH1R chimeras and subsequent point mutational analysis identified three divergent residues near the extracellular ends of trans-membrane helix 5 (TM5; Ser<sup>370</sup> and Val<sup>371</sup>, rat PTH1R) and TM6 (Leu<sup>427</sup>, rat PTH1R) that determine [Arg<sup>2</sup>]PTH-(1-34) signaling specificity (Fig. 2) (33).

The above functional studies on PTH1R mutants suggested a simple scheme for the ligand-receptor interaction in which the carboxy-terminal region of PTH-(1-34) interacts with the amino-terminal extracellular domain of the receptor and that the amino-terminal portion of the ligand interacts with the receptor region comprising the membrane-spanning helices and extracellular loops. As summarized below, subsequent functional studies with other receptor variants

that have been evaluated with different PTH or PTHrP analogs, as well as recent photoaffinity cross-linking studies, have supported this scheme.

The type 2 PTH receptor (PTH2R) is 51% identical to the type 1 receptor and responds fully to PTH, but not at all, or very poorly, to PTHrP (113). The biological importance of the PTH2R, which is expressed in only a few tissues (112, 113), is currently unknown, but its unique ligand specificity has provided additional clues regarding ligand-receptor interaction sites. The analysis of PTH/PTHrP hybrid ligands revealed that Phe<sup>23</sup> in PTHrP (Trp in PTH) prevents high-affinity binding to the PTH2R (36) and that the presence of His<sup>5</sup> in PTHrP (Ile in PTH) blocked cAMP signaling (Fig. 1A) (6, 36). Subsequent studies in which single amino acids of the PTH2R were replaced with the corresponding residues of the PTH1R led to the identification of several new determinants of ligand specificity. In particular, Ile<sup>244</sup>, Tyr<sup>318</sup>, and Cys<sup>397</sup>, which are located in the PTH2R at the extracellular ends of TM3, TM5, or TM7, respectively (replaced by Leu<sup>289</sup>, Ile<sup>363</sup>, and Tyr<sup>443</sup> in the human PTH1R, respectively), determined the receptor's ability to increase cAMP accumulation in response to PTHrP (Fig. 2) (8, 110). Furthermore, the data on Ile<sup>244</sup> and Tyr<sup>318</sup> suggested that these residues functionally interact with residue 5 of the ligand (8).

a) Candidate interaction sites identified by mutagenesis:			
<u>receptor</u>		<u>ligand</u>	
T <sup>33</sup> , Q <sup>37</sup>	--	(7-34) region	
W <sup>437</sup> , Q <sup>440</sup>	--	Ser <sup>1</sup> & Val <sup>2</sup>	
S <sup>370</sup> , I <sup>371</sup> , T <sup>427</sup>	--	Arg <sup>2</sup>	
b) Determinants of PTH-2 receptor ligand selectivity:			
<u>receptor</u>		<u>ligand</u>	
I <sup>244</sup> , Y <sup>318</sup>	--	His <sup>5</sup> in PTHrP,	
(L <sup>289</sup> , I <sup>363</sup> in P1R)	--	Ile <sup>5</sup> (PTH)	
c) Intramolecular interaction identified by mutagenesis:			
R <sup>233</sup> (TM2)	--	Q <sup>451</sup> (TM7)	
d) Residues identified by crosslinking:			
<u>receptor residue(s)</u>		<u>ligand</u>	
(23-40)	--	Bpa <sup>23</sup>	
R <sup>186</sup>	--	Bpz <sup>13</sup>	
M <sup>425</sup>	--	Bpa <sup>1</sup>	
e) Disease-associated receptor mutations:			
Blomstrand's:		P <sup>132</sup> ->L	
Jansen's (activating)		H <sup>223</sup> ->R, T <sup>410</sup> ->P,	
		I <sup>458</sup> ->R.	

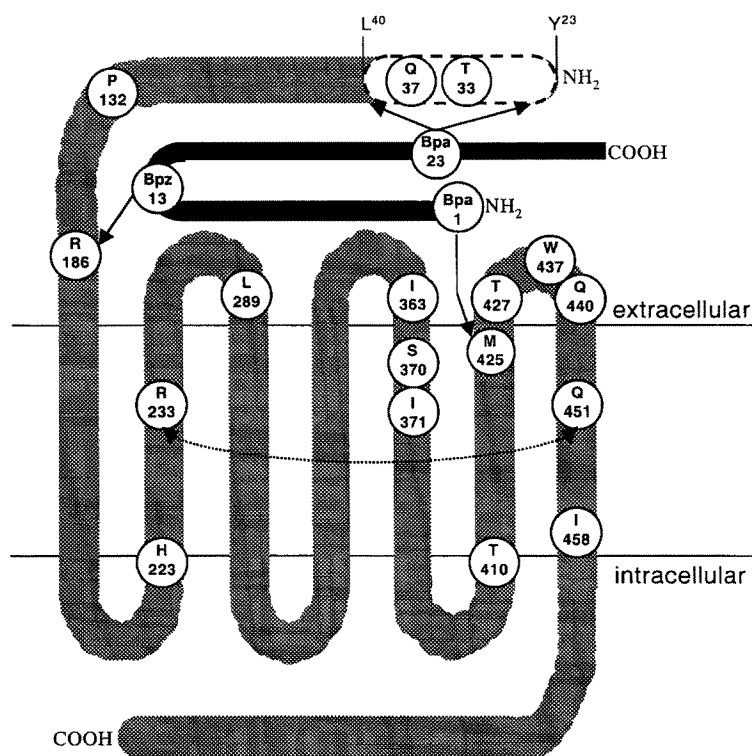


Fig. 2. Key sites in the PTH receptor and PTH or PTHrP ligand. Schematic shows some of the sites in the PTH-1 receptor (thick gray-shaded line) and PTH (or PTHrP) ligand (narrower solid line) that have been found by mutagenesis or photochemical cross-linking methods to have potentially important roles in ligand binding or receptor function. Receptor amino acids and position number correspond to the human PTH-1 receptor sequence. The three positions in the ligand at which photoreactive benzophenone groups have been introduced for cross-linking analyses are indicated. Corresponding receptor amino acids or region (Tyr<sup>23</sup>-Met<sup>41</sup> for the benzoylphenylalanine-23 analog) that were identified as probable sites of cross-linking are indicated by the connecting arrows.

In addition to these sites, it is likely that other receptor domains, including the amino-terminal extracellular region, play a role in conferring complete ligand selectivity to the PTH2R (8, 19, 110).

The general mode of ligand-receptor interaction suggested from the above studies on PTH receptors may well be used by the other class II peptide hormone receptors. In support of this notion, chimeras formed between the PTH1R and the calcitonin receptor selectively responded to PTH/calcitonin hybrid ligands, such that the COOH-terminal portion of the hybrid ligand corresponded to the NH<sub>2</sub>-terminal domain of the receptor, and the NH<sub>2</sub>-terminal portion of the ligand corresponded to the body of the receptor (7). Additional studies on chimeras formed between the receptors for glucagon, secretin, and vasoactive intestinal peptide are also consistent with this hypothesis (8, 19, 41, 42, 99, 109).

The use of photoreactive PTH and PTHrP analogs as chemical cross-linking probes is providing a complementary approach to the mapping of ligand-receptor interactions by mutational methods. The peptide analogs that have been used most successfully for this purpose contain the photoreactive benzophenone moiety at sites tolerant to the modification. For example, Chorev and coworkers (2) showed that a PTH-(1-34) analog having the benzophenone functional group attached to the  $\epsilon$ -amino group of lysine-13 cross-linked to an eight-amino acid receptor fragment located just amino-terminal of TM1, and mutation of Arg<sup>186</sup> in this segment prevented the formation of the cross-link (Fig. 2). These results suggest that the side chains of Lys<sup>13</sup> and Arg<sup>186</sup> come within a few angstroms of each other (2). In another study by the same group, a PTH-(1-34) analog containing benzoylphenylalanine (Bpa) in place of alanine-1 covalently cross-linked to a receptor segment in the COOH-terminal portion of TM6 containing Met<sup>414</sup> and Met<sup>425</sup>, and mutation at Met<sup>425</sup> abolished the cross-link (11). Interestingly, Met<sup>425</sup> is close to the residues that were previously shown to determine selectivity for [Arg<sup>2</sup>]PTH-(1-34) (33) (Fig. 2).

A third cross-linking site has been identified by our group using an analog of PTHrP-(1-36) containing Bpa at position 23, in place of the native phenylalanine. In this case, the reactive site was mapped to an 18-amino acid segment at the extreme amino terminus of the receptor (residues 23-40) (72) (Fig. 1). Subsequent alanine-scanning mutagenesis of this region revealed two amino acid residues, Thr<sup>33</sup> and Gln<sup>37</sup>, that contribute functional binding interactions to the (7-34) portion of PTH (72).

So far, there has been good correlation between the mutational analyses performed on the PTH receptors and the cross-linking data. As these parallel studies progress, they should help to refine and constrain the model of the ligand-receptor interaction. Recently, Miller and colleagues (21) showed that a secretin analog containing Bpa at position 22 cross-linked to the distal NH<sub>2</sub>-terminal region of the secretin receptor, consistent with the findings in the PTH1R. Thus it seems likely that there will be considerable similarity in the ligand

interaction models developed for the different class II GPCRs.

A ligand-induced conformational shift in the portion of the receptor containing the seven membrane-spanning helices and connecting loops is thought to be at the heart of the signal transduction mechanism (25). However, for most GPCRs, this process is still poorly understood. Turner and coworkers (108, 109) found evidence that neighboring polar residues in the second transmembrane helix of the PTH1R and the secretin receptor modulate signaling responsiveness to agonist ligands (108), and the authors also obtained evidence for a role of TM2 in determining ligand selectivity (109). Scanning mutagenesis of the PTH1R identified functionally important segments in each of the three extracellular loops, and point mutational analysis of the third extracellular loop showed that changes at Trp<sup>437</sup> and Gln<sup>440</sup> impaired binding of PTH-(1-34), but not of PTH-(3-34) (64). Similar defects in PTH-(1-34) binding but not PTH-(3-34) binding occurred with point mutations at Arg<sup>233</sup> near the middle of TM2 and at Gln<sup>451</sup> in the middle of TM7 (Fig. 2). Such results imply that the membrane-spanning helices and the connecting extracellular loops form a part of the ligand-binding pocket that recognizes residues 1 and 2 of the ligand. Interestingly, when the mutations at Arg<sup>233</sup> and Gln<sup>451</sup> were combined, binding of PTH-(1-34) was restored, but cAMP signaling was greatly diminished (34). This result suggests that helix 2 and helix 7 may interact, which is consistent with topological models derived from sequence alignment data (22), and that these interactions play a role in transmembrane signaling. Further insights into the conformational changes involved in the receptor signaling mechanism may come from studies on constitutively active receptors and inverse agonists (see below).

#### ROLE OF THE PTH1R IN KIDNEY

The mRNA transcript encoding the PTH1R is abundantly expressed in kidney and bone, where it is essential for the regulation of mineral ion homeostasis (65, 106, 111). In kidney, PTH stimulates the reabsorption of calcium in the distal nephron, inhibits the reabsorption of phosphate and bicarbonate in the proximal tubules, and enhances the synthesis of 1,25(OH)<sub>2</sub>D<sub>3</sub> in proximal tubular cells (15, 23, 27, 32, 76). The PTH1R mRNA can be found by *in situ* hybridization techniques in the convoluted and straight portion of the proximal tubules, in the cortical portion of thick ascending limbs, and in the distal convoluted tubules, which coincides with the previously established sites of renal PTH actions (65).

The PTH1R mRNA is transcribed from at least three distinct promoters that give rise to several splice variants differing in the 5' region (53, 75). In humans, most kidney-specific PTH1R transcripts are derived from a promoter located just upstream of the exon encoding the predicted signal peptide (9). Interestingly, Northern blot analysis has shown that glomerular podocytes express a 4.0-kb transcript instead of the typical 2.4-kb PTH1R mRNA transcript found in most other tissues (65), suggesting the use of a far-upstream

promoter; and PTH1R protein has been identified in podocytes by immunohistochemical analysis (3). Although the biological significance of these variations in PTH1R gene expression remains uncertain, the demonstration of PTH binding and PTH-mediated second messenger accumulation in freshly isolated glomeruli and in some podocyte cell lines is consistent with a role for PTH (and/or PTHrP) in glomerular function (74, 96).

#### ROLE OF THE PTH1R IN DEVELOPMENT

The PTH1R is highly expressed in the prehypertrophic chondrocytes of metaphyseal growth plates (66, 67), and in these cells it mediates the autocrine/paracrine actions of PTHrP. By acting through a complex signaling network involving the hedgehog, Wnt, and BMP family of proteins, PTHrP slows the differentiation of growth plate chondrocytes and prevents them from entering the hypertrophic stage. This action of PTHrP allows the bone to grow and elongate normally (57, 63, 116, 118).

Similar to the expression patterns of PTHrP, the PTH1R mRNA is expressed in a variety of other fetal and adult organs (65, 106, 111), and the biological roles mediated by the receptor in these tissues are beginning to be unraveled. For example, PTHrP and the PTH1R have been shown to regulate branching morphogenesis in the mammary gland (121), and other studies have suggested a role for these proteins in the development of skin, hair, pancreas, and teeth (40, 82, 90, 115, 120, 121).

#### PTH1R MUTATIONS ARE THE MOST PLAUSIBLE CAUSE OF TWO RARE GENETIC DISORDERS IN HUMANS

Because of the importance of the PTH1R in regulating blood calcium levels and skeletal development, it was predicted that mutations in its gene would be associated with severe abnormalities in mineral ion homeostasis or endochondral bone formation (57, 63, 116). These considerations led to the identification of the underlying molecular defects in two rare genetic disorders.

Three different heterozygous PTH1R mutations have been found in genomic DNA of patients with Jansen's metaphyseal chondrodysplasia, a rare autosomal dominant disorder that is characterized by short-limbed dwarfism due to an abnormal regulation of endochondral bone formation, severe PTH- and PTHrP-independent hypercalcemia, and increased bone turnover (87–89). Each of these mutations was shown to cause constitutive, agonist-independent activation of the receptor. When expressed in COS-7 cells, human PTH1Rs carrying either the His<sup>223</sup> → Arg, the Thr<sup>410</sup> → Pro, or the Ile<sup>458</sup> → Arg mutation showed five- to eightfold increases in basal intracellular cAMP levels, in comparison with cells expressing the wild-type receptor. Basal inositol trisphosphate levels were indistinguishable for cells expressing wild-type or mutant receptors. Analogs of PTH and PTHrP that had been developed earlier as competitive antagonists of PTH-(1–34) action, such as [Leu<sup>11</sup>,D-Trp<sup>12</sup>]PTHrP-(7–34), function as inverse ago-

nists with the constitutively active PTH1Rs; that is, they dose-dependently reduce cAMP levels in cells expressing the mutant receptors (35). When the human PTH1R with the His<sup>223</sup> → Arg mutation was expressed as a transgene in mice under the control of the rat  $\alpha$ 1(II) collagen promoter, the growth plates of the animals showed a significant deceleration of chondrocyte differentiation (90). This phenotype was similar to that of mice expressing the PTHrP gene under the control of the same promoter (118) and was the mirror image of that found in mice having "knock-out" mutations of either the PTHrP gene or the PTH1R gene (57, 63). These in vitro and in vivo findings with mutant constitutively active PTH1Rs provided a plausible explanation for the skeletal abnormalities seen in patients with Jansen's disease.

Mutations that impair PTH1R function were recently identified in two unrelated cases of Blomstrand's chondrodysplasia, a rare autosomal recessive disorder characterized by early lethality, advanced bone maturation with accelerated chondrocyte differentiation, and probable defects in mineral ion homeostasis (14). The skeletal changes seen in the affected infants are similar to those observed in mice homozygous for the null allele of the PTHrP or PTH1R gene (58, 63) and are consistent with the presence of homozygous or compound heterozygous mutations in the PTH1R gene. The mutation found in the PTH1R gene of one of the affected infants altered the splicing pattern of the maternally derived mRNA, such that 11 amino acids (residues 373–383) in TM5 were deleted (50). For still unknown reasons, the paternal allele was not expressed, resulting in a "null" phenotype for PTH1R expression. In another case of Blomstrand's disease that resulted from a consanguineous marriage, a homozygous point mutation in the PTH1R was found that substituted leucine for the highly conserved proline-132 in the receptor's amino-terminal domain (Fig. 2) (56, 123). Both the 11-amino acid deletion and the Pro<sup>132</sup> → Leu mutation impaired binding of PTH and PTHrP and markedly reduced responsiveness to these ligands in cAMP accumulation assays.

#### PSEUDOHYPOPARATHYROIDISM TYPE 1B IS NOT CAUSED BY MUTATIONS IN THE PTH1R GENE

Pseudohypoparathyroidism type 1b (PHP-Ib) is a rare disorder characterized by hypocalcemia and hyperphosphatemia, which is caused by resistance toward PTH. Unlike patients affected by PHP-Ia, which show reduced activity of the stimulatory G $\alpha$  protein due to a variety of mutations in its gene (*GNAS1*), individuals affected by PHP-Ib show no resistance toward other hormones and no associated developmental abnormalities (68, 114, 117). Because of this selective resistance toward a single hormone, PHP-Ib was initially thought to be caused by inactivating mutations in the PTH1R gene (94, 95). However, such mutations were excluded in a considerable number of PHP-Ib patients for the coding and noncoding exons (10, 29, 91) and at the mRNA level (30, 100). Consistent with the conclusion that there are no structural abnormalities in the



PTH1R, individuals with PHP-Ib frequently show normal osseous response to PTH or even biochemical and radiological evidence for increased osteoclastic resorption, indicating that the PTH-dependent actions on osteoblasts are not impaired (68, 78, 114). Moreover, PHP-Ib patients typically show no abnormalities in growth plate development and thus show normal longitudinal growth, indicating that the PTHrP-dependent regulation of chondrocyte growth and differentiation is normal. Recently, a genome-wide search using genomic DNA from numerous affected and unaffected individuals of four unrelated kindreds led to the identification of a locus on the telomeric end of chromosome 20q, which contains at least portions of the *GNAS1* gene. These findings suggest the possibility that PHP-Ib is caused by a defect in a tissue- or cell-specific enhancer or promoter of the *GNAS1* gene, or an as yet unidentified gene in the same chromosomal region (54).

In summary, significant progress has been made in understanding the role of the common PTH/PTHrP receptor, the PTH1R, in mammalian biology, particularly with regard to its normal role in chondrocyte growth and development, and its pathological role in two rare genetic disorders in humans. Amino acid residues in the PTH1R and PTH2R that are likely to be important for ligand-receptor interaction and for signal transduction have been identified through mutagenesis methods and through photoaffinity cross-linking techniques. Although these studies have provided new insights into the mode of ligand-receptor interaction, there is still much that needs to be learned about this complex process. Furthermore, it will be important to investigate the biological importance of the PTH2R, to search for novel receptors that may be selective for different portions of PTH or PTHrP, and to identify new peptide ligands that act upon these systems.

Present address of M. Mannstadt: Medizinische Poliklinik, Ludwig-Maximilians-Universität, Munich, Germany 80336.

Address for reprint requests and other correspondence: H. Jüppner, Endocrine Unit, Department of Medicine and Pediatrics, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114. (E-mail: jueppner@helix.mgh.harvard.edu).

## REFERENCES

1. Abou-Samra, A.-B., S. Uneno, H. Jüppner, H. Keutmann, J. T. Potts, Jr., G. V. Segre, and S. R. Nussbaum. Non-homologous sequences of parathyroid hormone and the parathyroid hormone related peptide bind to a common receptor on ROS 17/2.8 cells. *Endocrinology* 125: 2215–2217, 1989.
2. Adams, A., A. Bisello, M. Choev, M. Rosenblatt, and L. Suva. Arginine 186 in the extracellular N-terminal region of the human parathyroid hormone 1 receptor is essential for contact with position 13 of the hormone. *Mol. Endocrinol.* 12: 1673–1683, 1998.
3. Amizuka, N., H. S. Lee, M. Y. Khan, A. Arazani, H. Warhawsky, G. N. Hendy, H. Ozawa, J. H. White, and D. Goltzman. Cell-specific expression of the parathyroid hormone (PTH)/PTH-related peptide receptor gene in kidney from kidney-specific and ubiquitous promoters. *Endocrinology* 138: 469–481, 1997.
4. Barden, J., R. Cuthbertson, W. Jia-Zhen, J. Moseley, and B. Kemp. Solution structure of parathyroid hormone related protein (residues 1–34) containing an Ala substituted for an Ile in position 15 (PTHrP[Ala<sup>15</sup>]-1–34). *J. Biol. Chem.* 273: 29572–29578, 1997.
5. Barden, J. A., and B. E. Kemp. Stabilized NMR structure of the hypercalcemia of malignancy peptide PTHrP[Ala-26](1–34)amide. *Biochim. Biophys. Acta* 1208: 256–262, 1994.
6. Behar, V., C. Nakamoto, Z. Greenberg, A. Bisello, L. J. Suva, M. Rosenblatt, and M. Choev. Histidine at position 5 is the specificity “switch” between two parathyroid hormone receptor subtypes. *Endocrinology* 137: 4217–4224, 1996.
7. Bergwitz, C., T. J. Gardella, M. R. Flannery, J. T. Potts, H. M. Kronenberg, S. R. Goldring, and H. Jüppner. Full activation of chimeric receptors by hybrids between parathyroid hormone and calcitonin. *J. Biol. Chem.* 271: 26469–26472, 1996.
8. Bergwitz, C., S. A. Jusseaume, M. D. Luck, H. Jüppner, and T. J. Gardella. Residues in the membrane-spanning and extracellular regions of the parathyroid hormone (PTH)-2 receptor determine signaling selectivity for PTH and PTH-related peptide. *J. Biol. Chem.* 272: 28861–28868, 1997.
9. Bettoun, J. D., M. Minagawa, G. N. Hendy, L. C. Alpert, C. G. Goodyer, D. Goltzman, and J. H. White. Developmental upregulation of the human parathyroid hormone (PTH)/PTH-related peptide receptor gene expression from conserved and human-specific promoters. *J. Clin. Invest.* 102: 958–967, 1998.
10. Bettoun, J. D., M. Minagawa, M. Y. Kwan, H. S. Lee, T. Yasuda, G. N. Hendy, D. Goltzman, and J. H. White. Cloning and characterization of the promoter regions of the human parathyroid hormone (PTH)/PTH-related peptide receptor gene: analysis of deoxyribonucleic acid from normal subjects and patients with pseudohypoparathyroidism type Ib. *J. Clin. Endocrinol. Metab.* 82: 1031–1040, 1997.
11. Bisello, A., A. E. Adams, D. F. Mierke, M. Pellegrini, M. Rosenblatt, L. J. Suva, and M. Choev. Parathyroid hormone-receptor interactions identified directly by photocross-linking and molecular modeling studies. *J. Biol. Chem.* 273: 22498–22505, 1998.
12. Blind, E., T. Bambino, Z. Huang, M. Bliziotis, and R. A. Nissenson. Phosphorylation of the cytoplasmic tail of the PTH/PTHrP receptor. *J. Bone Miner. Res.* 11: 578–586, 1996.
13. Blind, E., T. Bambino, and R. A. Nissenson. Agonist-stimulated phosphorylation of the G protein-coupled receptor for parathyroid hormone (PTH) and PTH-related protein. *Endocrinology* 136: 4271–4277, 1995.
14. Blomstrand, S., I. Claesson, and J. Sävje-Söderbergh. A case of lethal congenital dwarfism with accelerated skeletal maturation. *Pediatr. Radiol.* 15: 141–143, 1985.
15. Bringham, F. R. Calcium and phosphate distribution, turnover, and metabolic actions. In: *Endocrinology*, edited by L. J. DeGroot. Philadelphia, PA: Saunders, 1989, p. 805–843.
16. Brown, E. M., G. Gamba, D. Riccardi, M. Lombardi, R. Butters, O. Kifor, A. Sun, M. A. Hediger, J. Lytton, and S. C. Hebert. Cloning and characterization of an extracellular Ca<sup>2+</sup>-sensing receptor from bovine parathyroid. *Nature* 366: 575–580, 1993.
17. Caulfield, M. P., and M. Rosenblatt. Parathyroid hormone-receptor interactions. *Trends Endocrinol. Metab.* 2: 164–168, 1990.
18. Cheng, L., and B. Sacktor. Sodium gradient-dependent phosphate transport in renal brush border vesicles. *J. Biol. Chem.* 256: 1556–1564, 1981.
19. Clark, J. A., T. I. Bonner, A. S. Kim, and T. B. Usdin. Multiple regions of ligand discrimination revealed by analysis of chimeric parathyroid hormone 2 (PTH2) and PTH/PTH-related peptide (PTHrP) receptors. *Mol. Endocrinol.* 12: 193–206, 1998.
20. Cohen, F. E., G. J. Stewler, M. S. Bradley, M. Carlquist, M. Nilsson, M. Ericsson, T. L. Ciardelli, and R. A. Nissenson. Analogues of parathyroid hormone modified at positions 3 and 6: effects on receptor binding and activation of adenylyl cyclase in kidney and bone. *J. Biol. Chem.* 266: 1997–2004, 1991.
21. Dong, M., Y. Wang, D. Pinon, E. Hadac, and L. Miller. Demonstration of a direct interaction between residue 22 in the carboxyl-terminal half of secretin and the amino-terminal tail of the secretin receptor using photoaffinity labeling. *J. Biol. Chem.* 274: 903–909, 1999.



22. Donnelly, D. The arrangement of the transmembrane helices in the secretin receptor family of G-protein-coupled receptors. *FEBS Lett.* 409: 431–436, 1997.
23. Drezner, M. K. Phosphorus homeostasis and related disorders. In: *Principles in Bone Biology*, edited by J. P. Bilezikian, L. G. Raisz, and G. A. Rodan. New York: Academic, 1996, p. 263–276.
24. Everhart-Caye, M., S. E. Inzucchi, J. Guinness-Henry, M. A. Mitnick, and A. F. Stewart. Parathyroid hormone (PTH)-related protein(1–36) is equipotent to PTH-(1–34) in humans. *J. Clin. Endocrinol. Metab.* 81: 199–208, 1996.
25. Farrens, D. L., C. Altenbach, K. Yang, W. L. Hubbell, and H. G. Khorana. Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. *Science* 274: 768–770, 1996.
26. Fraher, L. J., A. B. Hodsman, K. Jonas, D. Saunders, C. I. Rose, J. E. Henderson, G. N. Hendy, and D. Goltzman. A comparison of the in vivo biochemical responses to exogenous parathyroid hormone-(1–34) [PTH-(1–34)] and PTH-related peptide-(1–34) in man. *J. Clin. Endocrinol. Metab.* 75: 417–423, 1992.
27. Fraser, D. R., and E. Kodicek. Regulation of 25-hydroxycholecalciferol-1-hydroxylase activity in kidney by parathyroid hormone. *Nature* 241: 163–166, 1973.
28. Friedman, P. A., F. A. Gesek, P. Morley, J. F. Whitfield, and G. E. Willick. Cell-specific signaling and structure-activity relations of parathyroid hormone analogs in mouse kidney cells. *Endocrinology* 140: 301–309, 1999.
29. Fukumoto, S., M. Suzawa, T. Kikuchi, T. Matsumoto, S. Kato, and T. Fujita. Cloning and characterization of kidney-specific promoter of human PTH/PTHrP receptor gene: absence of mutation in patients with pseudohypoparathyroidism type Ib. *Mol. Cell. Endocrinol.* 141: 41–47, 1998.
30. Fukumoto, S., M. Suzawa, Y. Takeuchi, K. Nakayama, Y. Kodama, E. Ogata, and T. Matsumoto. Absence of mutations in parathyroid hormone (PTH)/PTH-related protein receptor complementary deoxyribonucleic acid in patients with pseudohypoparathyroidism type Ib. *J. Clin. Endocrinol. Metab.* 81: 2554–2558, 1996.
31. Gagnon, L., H. Jouishomme, J. F. Whitfield, J. P. Durkin, S. MacLean, W. Neugebauer, G. Willick, R. H. Rixon, and B. Chakravarthy. Protein kinase C-activating domains of parathyroid hormone-related protein. *J. Bone Miner. Res.* 8: 497–503, 1993.
32. Garabedian, M., M. F. Holick, H. F. Deluca, and I. T. Boyle. Control of 25-hydroxycholecalciferol metabolism by parathyroid glands. *Proc. Natl. Acad. Sci. USA* 69: 1673–1676, 1972.
33. Gardella, T. J., H. Jüppner, A. K. Wilson, H. T. Keutmann, A. B. Abou-Samra, G. V. Segre, F. R. Bringhurst, J. T. Potts, Jr., S. R. Nussbaum, and H. M. Kronenberg. Determinants of [Arg<sup>2</sup>]PTH-(1–34) binding and signaling in the transmembrane region of the parathyroid hormone receptor. *Endocrinology* 135: 1186–1194, 1994.
34. Gardella, T. J., M. D. Luck, M.-H. Fan, and C. W. Lee. Transmembrane residues of the parathyroid hormone (PTH)/PTH-related peptide receptor that specifically affect binding and signaling by agonist ligands. *J. Biol. Chem.* 271: 12820–12825, 1996.
35. Gardella, T. J., M. D. Luck, G. S. Jensen, E. Schipani, J. T. Potts, Jr., and H. Jüppner. Inverse agonism of amino-terminally truncated parathyroid hormone (PTH) and PTH-related peptide (PTHrP) analogs revealed with constitutively active mutant PTH/PTHrP receptors linked to Jansen's metaphyseal chondrodysplasia. *Endocrinology* 137: 3936–3941, 1996.
36. Gardella, T. J., M. D. Luck, G. S. Jensen, T. B. Usdin, and H. Jüppner. Converting parathyroid hormone-related peptide (PTHrP) into a potent PTH-2 receptor agonist. *J. Biol. Chem.* 271: 19888–19893, 1996.
37. Gardella, T. J., M. D. Luck, A. K. Wilson, H. T. Keutmann, S. R. Nussbaum, J. T. Potts, Jr., and H. M. Kronenberg. Parathyroid hormone (PTH)/PTH-related peptide hybrid peptides reveal functional interactions between the 1–14 and 15–34 domains of the ligand. *J. Biol. Chem.* 270: 6584–6588, 1995.
38. Goltzman, D., A. Peytremann, E. Callahan, G. W. Tregear, and J. T. Potts, Jr. Analysis of the requirements for parathyroid hormone action in renal membranes with the use of inhibiting analogues. *J. Biol. Chem.* 250: 3199–3203, 1975.
39. Guo, J., A. Iida-Klein, X. Huang, A. B. Abou-Samra, G. V. Segre, and F. R. Bringhurst. Parathyroid hormone (PTH)/PTH-related peptide receptor density modulates activation of phospholipase C and phosphate transport by PTH in LLC-PK1 cells. *Endocrinology* 136: 3884–3891, 1995.
40. Holick, M. F., S. Ray, T. C. Chen, X. Tian, and K. S. Persons. A parathyroid hormone antagonist stimulates epidermal proliferation and hair growth in mice. *Proc. Natl. Acad. Sci. USA* 91: 8014–8016, 1994.
41. Holtmann, M., E. Hadac, and L. Miller. Critical contributions of amino-terminal extracellular domains in agonist binding and activation of secretin and vasoactive intestinal polypeptide receptors. *J. Biol. Chem.* 270: 14394–14398, 1995.
42. Holtmann, M. H., E. M. Hadac, C. D. Ulrich, and L. J. Miller. Molecular basis and species specificity of high affinity binding of vasoactive intestinal polypeptide by the rat secretin receptor. *J. Pharmacol. Exp. Ther.* 279: 555–560, 1996.
43. Horiuchi, N., M. P. Caulfield, J. E. Fisher, M. E. Goldman, R. L. McKee, J. E. Reagan, J. J. Levy, R. F. Nutt, S. B. Rodan, T. L. Schofield, T. L. Clemens, and M. Rosenblatt. Similarity of synthetic peptide from human tumour to parathyroid hormone in vivo and in vitro. *Science* 238: 1566–1568, 1987.
44. Horiuchi, N., M. F. Holick, J. T. Potts, Jr., and M. Rosenblatt. A parathyroid hormone inhibitor in vivo: design and biologic evaluation of a hormone analog. *Science* 220: 1053–1055, 1983.
45. Huang, Z., Y. Chen, S. Pratt, T.-H. Chen, T. Bambino, R. Nissenson, and D. Shoback. The NH<sub>2</sub>-terminal region of the third intracellular loop of the parathyroid hormone (PTH)/PTH-related peptide receptor is critical for coupling to cAMP and inositol phosphate/Ca<sup>2+</sup> signal transduction pathways. *J. Biol. Chem.* 271: 33382–33389, 1996.
46. Iida-Klein, A., J. Guo, M. Takemura, M. T. Drake, J. T. Potts, Jr., A. Abou-Samra, F. R. Bringhurst, and G. V. Segre. Mutations in the second cytoplasmic loop of the rat parathyroid hormone (PTH)/PTH-related protein receptor result in selective loss of PTH-stimulated phospholipase C activity. *J. Biol. Chem.* 272: 6882–6889, 1997.
47. Iida-Klein, A., J. Guo, L. Y. Xie, H. Jüppner, J. T. Potts, Jr., H. M. Kronenberg, F. R. Bringhurst, A. B. Abou-Samra, and G. V. Segre. Truncation of the carboxyl-terminal region of the parathyroid hormone (PTH)/PTH-related peptide receptor enhances PTH stimulation of adenylate cyclase but not phospholipase C. *J. Biol. Chem.* 270: 8458–8465, 1995.
48. Inomata, N., M. Akiyama, N. Kubota, and H. Jüppner. Characterization of a novel PTH-receptor with specificity for the carboxyl-terminal region of PTH-(1–84). *Endocrinology* 136: 4732–4740, 1995.
49. Ji, T. H., M. Grossmann, and I. Ji. G protein-coupled receptors. I. Diversity of receptor-ligand interactions. *J. Biol. Chem.* 273: 17299–17302, 1998.
50. Jobert, A. S., P. Zhang, A. Couvineau, J. Bonaventure, J. Roume, M. LeMerrer, and C. Silve. Absence of functional receptors parathyroid hormone and parathyroid hormone-related peptide in Blomstrand chondrodysplasia. *J. Clin. Invest.* 102: 34–40, 1998.
51. Jouishomme, H., J. F. Whitfield, B. Chakravarthy, J. P. Durkin, L. Gagnon, R. J. Isaacs, S. MacLean, W. Neugebauer, G. Willick, and R. H. Rixon. The protein kinase-C activation domain of the parathyroid hormone. *Endocrinology* 130: 53–59, 1992.
52. Jouishomme, H., J. F. Whitfield, L. Gagnon, S. Maclean, R. Isaacs, B. Chakravarthy, J. Durkin, W. Neugebauer, G. Willick, and R. H. Rixon. Further definition of the protein kinase C activation domain of the parathyroid hormone. *J. Bone Miner. Res.* 9: 943–949, 1994.
53. Joun, H., B. Lanske, M. Karperien, F. Qian, L. Defize, and A. Abou-Samra. Tissue-specific transcription start sites and alternative splicing of the parathyroid hormone (PTH)/PTH-related peptide (PTHrP) receptor gene: a new PTH/PTHrP

- receptor splice variant that lacks the signal peptide. *Endocrinology* 138: 1742–1749, 1997.
54. Jüppner, H., E. Schipani, M. Bastepe, D. E. C. Cole, M. L. Lawson, M. Mannstadt, G. N. Hendy, H. Plotkin, H. Koshiyama, T. Koh, J. D. Crawford, B. R. Olsen, and M. Vikkula. The gene responsible for pseudohypoparathyroidism type 1b is paternally imprinted and maps in four unrelated kindreds to chromosome 20q13.3. *Proc. Natl. Acad. Sci. USA* 95: 11798–11803, 1998.
  55. Jüppner, H., E. Schipani, F. R. Bringhurst, I. McClure, H. T. Keutmann, J. T. Potts, Jr., H. M. Kronenberg, A. B. Abou-Samra, G. V. Segre, and T. J. Gardella. The extracellular, amino-terminal region of the parathyroid hormone (PTH)/PTH-related peptide (PTHrP) receptor determines the binding affinity for carboxyl-terminal fragments of PTH-(1–34). *Endocrinology* 134: 879–884, 1994.
  56. Karaplis, A., B. He, M. Nguyen, I. Young, D. Semeraro, H. Ozawa, and N. Amizuka. Inactivating mutation in the human PTH receptor type 1 gene in blomstrand chondrodysplasia. *Endocrinology* 139: 5255–5258, 1998.
  57. Karaplis, A. C., A. Luz, J. Glowacki, R. Bronson, V. Tybulewicz, H. M. Kronenberg, and R. C. Mulligan. Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. *Genes Dev.* 8: 277–289, 1994.
  58. Karaplis, A. C., T. Yasuda, G. N. Hendy, D. Goltzman, and D. Banville. Gene encoding parathyroid hormone-like peptide: nucleotide sequence of the rat gene and comparison with the human homologue. *Mol. Endocrinol.* 4: 441–446, 1990.
  59. Kemp, B. E., J. M. Mosely, C. P. Rodda, P. R. Ebeling, R. E. H. Wettenhall, D. Stapleton, H. Diefenbach-Jagger, F. Ure, V. P. Michelangeli, H. A. Simmons, L. G. Raisz, and T. J. Martin. Parathyroid hormone-related protein of malignancy: active synthetic fragments. *Science* 238: 1568–1570, 1987.
  60. Kolakowski, L. F. J. GCRDB: a G-protein-coupled receptor database. *Receptors Channels* 2: 1–7, 1994.
  61. Kovacs, C. S., B. Lanske, J. L. Hunzelman, J. Guo, A. C. Karaplis, and H. M. Kronenberg. Parathyroid hormone-related peptide (PTHrP) regulates fetal placental calcium transport through a receptor distinct from the PTH/PTHrP receptor. *Proc. Natl. Acad. Sci. USA* 93: 15233–15238, 1996.
  62. Kronenberg, H. M., F. R. Bringhurst, S. Nussbaum, H. Jüppner, A. B. Abou-Samra, G. V. Segre, and J. T. Potts, Jr. Parathyroid hormone: biosynthesis, secretion, chemistry, and action. In: *Handbook of Experimental Pharmacology: Physiology and Pharmacology of Bone*, edited by G. R. Mundy and T. J. Martin. Heidelberg: Springer-Verlag, 1993, p. 185–201.
  63. Lanske, B., A. C. Karaplis, A. Luz, A. Vortkamp, A. Pirro, M. Karperien, L. H. K. Defize, C. Ho, R. C. Mulligan, A. B. Abou-Samra, H. Jüppner, G. V. Segre, and H. M. Kronenberg. PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth. *Science* 273: 663–666, 1996.
  64. Lee, C., M. D. Luck, H. Jüppner, J. T. Potts, Jr., H. M. Kronenberg, and T. J. Gardella. Homolog-scanning mutagenesis of the parathyroid hormone (PTH) receptor reveals PTH-(1–34) binding determinants in the third extracellular loop. *Mol. Endocrinol.* 9: 1269–1278, 1995.
  65. Lee, K., D. Brown, P. Urena, N. Ardaillou, R. Ardaillou, J. Deeds, and G. V. Segre. Localization of parathyroid hormone/parathyroid hormone-related peptide receptor mRNA in kidney. *Am. J. Physiol.* 270 (Renal Fluid Electrolyte Physiol. 39): F186–F191, 1996.
  66. Lee, K., J. D. Deeds, and G. V. Segre. Expression of parathyroid hormone-related peptide and its receptor messenger ribonucleic acid during fetal development of rats. *Endocrinology* 136: 453–463, 1995.
  67. Lee, K., B. Lanske, A. C. Karaplis, J. D. Deeds, H. Kohno, R. A. Nissenson, H. M. Kronenberg, and G. V. Segre. Parathyroid hormone-related peptide delays terminal differentiation of chondrocytes during endochondral bone development. *Endocrinology* 137: 5109–5118, 1996.
  68. Levine, M. A. Pseudohypoparathyroidism. In: *Principles of Bone Biology*, edited by J. P. Bilezikian, L. G. Raisz, and G. A. Rodan. New York: Academic, 1996, p. 853–876.
  69. Malecz, N., T. Bambino, M. Bencsik, and R. Nissenson. Identification of phosphorylation sites in the G protein-coupled receptor for parathyroid hormone. Receptor phosphorylation is not required for agonist-induced internalization. *Mol. Endocrinol.* 12: 1846–1856, 1998.
  70. Malmström, K., and H. Murer. Parathyroid hormone regulates phosphate transport in OK cells via an irreversible inactivation of a membrane protein. *FEBS Lett.* 216: 257–260, 1987.
  71. Mangin, M., A. C. Webb, B. E. Dreyer, J. T. Posillico, K. Ikeda, E. C. Weir, A. F. Stewart, N. H. Bander, L. Milstone, D. E. Barton, U. Francke, and A. E. Broadus. Identification of a cDNA encoding a parathyroid hormone-like peptide from a human tumor associated with humoral hypercalcemia of malignancy. *Proc. Natl. Acad. Sci. USA* 85: 597–601, 1988.
  72. Mannstadt, M., M. Luck, T. J. Gardella, and H. Jüppner. Evidence for a ligand interaction site at the amino-terminus of the PTH/PTHrP receptor from crosslinking and mutational studies. *J. Biol. Chem.* 273: 16890–16896, 1998.
  73. Marx, U., S. Austermann, P. Bayer, K. Adermann, A. Ejchart, H. Sticht, S. Walters, F. Schmid, R. Jaenicke, W. Forssmann, and P. Rosch. Structure of human parathyroid hormone 1–37 in solution. *J. Biol. Chem.* 270: 15194–15202, 1995.
  74. Massfelder, T., C. Saussine, U. Simeoni, B. Enanga, C. Jude, and J. J. Helwig. Evidence for adenylyl cyclase-dependent receptors for parathyroid hormone (PTH)-related protein in rabbit kidney glomeruli. *Life Sci.* 53: 875–878, 1993.
  75. McCuaig, K. A., H. Lee, J. C. Clarke, H. Assar, J. Horsford, and J. H. White. Parathyroid hormone/parathyroid hormone related peptide receptor gene transcripts are expressed from tissue-specific and ubiquitous promoters. *Nucleic Acids Res.* 23: 1948–1955, 1995.
  76. Morel, F., M. Imbert-Teboul, and D. Charbardes. Distribution of hormone-dependent adenylyl cyclase in the nephron and its physiological significance. *Annu. Rev. Physiol.* 43: 569–581, 1981.
  77. Moseley, J. M., M. Kubota, H. Diefenbach-Jagger, R. E. H. Wettenhall, B. E. Kemp, L. J. Suva, C. P. Rodda, P. R. Ebeling, P. J. Hudson, J. D. Zajac, and J. T. Martin. Parathyroid hormone-related protein purified from a human lung cancer cell line. *Proc. Natl. Acad. Sci. USA* 84: 5048–5052, 1987.
  78. Murray, T., E. Gomez Rao, M. M. Wong, J. P. Waddell, R. McBroom, C. S. Tam, F. Rosen, and M. A. Levine. Pseudohypoparathyroidism with osteitis fibrosa cystica: direct demonstration of skeletal responsiveness to parathyroid hormone in cells cultured from bone. *J. Bone Miner. Res.* 8: 83–91, 1993.
  79. Nudley, M. T., S. A. Parimi, and S. Harvey. Sequence analysis of hypothalamic parathyroid hormone messenger ribonucleic acid. *Endocrinology* 136: 5600–5607, 1995.
  80. Pellegrini, M., M. Royo, M. Rosenblatt, M. Chorev, and D. Mierke. Addressing the tertiary structure of human parathyroid hormone-(1–34). *J. Biol. Chem.* 273: 10420–10427, 1998.
  81. Pfister, M. F., I. Ruf, G. Stange, U. Ziegler, E. Lederer, J. Biber, and H. Murer. Parathyroid hormone leads to the lysosomal degradation of the renal type II Na/P<sub>i</sub> cotransporter. *Proc. Natl. Acad. Sci. USA* 95: 1909–1914, 1998.
  82. Philbrick, W. M., E. C. Weir, A. C. Karaplis, B. E. Dreyer, and A. E. Broadus. Rescue of the PTHrP-null mouse reveals multiple developmental defects. *J. Bone Miner. Res.* 11, Suppl. 1: P268, 1996.
  83. Pines, M., A. E. Adams, S. Stueckle, R. Bessalle, V. Rashty-Bhar, M. Chorev, M. Rosenblatt, and L. J. Suva. Generation and characterization of human kidney cell lines stably expressing recombinant human PTH/PTHrP receptor: lack of interaction with a COOH-terminal human PTH peptide. *Endocrinology* 135: 1713–1716, 1994.
  84. Potts, J. T., Jr., and H. Jüppner. Parathyroid hormone and parathyroid hormone-related peptide in calcium homeostasis, bone metabolism, and bone development: the proteins, their genes, and receptors. In: *Metabolic Bone Disease*, edited by L. V. Avioli and S. M. Krane. New York: Academic, 1997, p. 51–94.
  85. Qian, F., A. Leung, and A. Abou-Samra. Agonist-dependent phosphorylation of the parathyroid hormone/parathyroid hor-

- hormone-related peptide receptor. *Biochemistry* 37: 6240–6246, 1998.
86. Quinn, J. M., J. Elliott, M. T. Gillespie, and T. J. Martin. A combination of osteoclast differentiation factor and macrophage-colony stimulating factor is sufficient for both human and mouse osteoclast formation in vitro. *Endocrinology* 139: 4424–4427, 1998.
  87. Schipani, E., K. Kruse, and H. Jüppner. A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. *Science* 268: 98–100, 1995.
  88. Schipani, E., C. Langman, J. Hunzelman, M. Le Merrer, K. Y. Loke, M. J. Dillon, C. Silve, and H. Jüppner. A novel parathyroid hormone (PTH)/PTH-related peptide receptor mutation in Jansen's metaphyseal chondrodysplasia. *J. Clin. Endocrinol. Metab.* In press.
  89. Schipani, E., C. B. Langman, A. M. Parfitt, G. S. Jensen, S. Kikuchi, S. W. Kooh, W. G. Cole, and H. Jüppner. Constitutively activated receptors for parathyroid hormone and parathyroid hormone-related peptide in Jansen's metaphyseal chondrodysplasia. *N. Engl. J. Med.* 335: 708–714, 1996.
  90. Schipani, E., B. Lanske, J. Hunzelman, C. S. Kovacs, K. Lee, A. Pirro, H. M. Kronenberg, and H. Jüppner. Targeted expression of constitutively active PTH/PTHrP receptors delays endochondral bone formation and rescues PTHrP-less mice. *Proc. Natl. Acad. Sci. USA* 94: 13689–13694, 1997.
  91. Schipani, E., L. S. Weinstein, C. Bergwitz, A. Iida-Klein, X. F. Kong, M. Stuhmann, K. Kruse, M. P. Whyte, T. Murray, J. Schmidtke, C. van Dop, A. S. Brickman, J. D. Crawford, J. T. Potts, Jr., H. M. Kronenberg, A. B. Abou-Samra, G. V. Segre, and H. Jüppner. Pseudohypoparathyroidism type 1b is not caused by mutations in the coding exons of the human parathyroid hormone (PTH)/PTH-related peptide receptor gene. *J. Clin. Endocrinol. Metab.* 80: 1611–1621, 1995.
  92. Segre, G. V. Receptors for parathyroid hormone and parathyroid hormone-related protein. In: *Principles in Bone Biology*, edited by J. P. Bilezikian, L. G. Raisz, and G. A. Rodan. New York: Academic, 1996, p. 377–403.
  93. Segre, G. V., and S. R. Goldring. Receptors for secretin, calcitonin, parathyroid hormone (PTH)/PTH-related peptide, vasoactive intestinal peptide, glucagon-like peptide 1, growth hormone-releasing hormone, and glucagon belong to a newly discovered G protein-linked receptor family. *Trends Endocrinol. Metab.* 4: 309–314, 1993.
  94. Silve, C., A. Santora, N. Breslau, A. Moses, and A. Spiegel. Selective resistance to parathyroid hormone in cultured skin fibroblasts from patients with pseudohypoparathyroidism type 1b. *J. Clin. Endocrinol. Metab.* 62: 640–644, 1986.
  95. Silve, C., F. Suarez, A. El Hessni, A. Loiseau, A. M. Graulet, and J. Gueris. The resistance to parathyroid hormone of fibroblasts from some patients with type 1b pseudohypoparathyroidism is reversible with dexamethasone. *J. Clin. Endocrinol. Metab.* 71: 631–638, 1990.
  96. Sraer, J., J. D. Sraer, D. Chansel, H. Jueppner, R. D. Hesch, and R. Ardaillou. Evidence for glomerular receptors for parathyroid hormone. *Am. J. Physiol.* 235 (Renal Fluid Electrolyte Physiol. 4): F96–F103, 1978.
  97. Stewart, A. F., R. Horst, L. J. Deftos, E. C. Cadman, R. Lang, and A. E. Broadus. Biochemical evaluation of patients with cancer-associated hypercalcemia. Evidence for humoral and non-humoral groups. *N. Engl. J. Med.* 303: 1377–1381, 1980.
  98. Strewler, G. J., P. H. Stern, J. W. Jacobs, J. Eveloff, R. F. Klein, S. C. Leung, M. Rosenblatt, and R. A. Nissenson. Parathyroid hormone-like protein from human renal carcinoma cells. Structural and functional homology with parathyroid hormone. *J. Clin. Invest.* 80: 1803–1807, 1987.
  99. Stroop, S. D., R. E. Kuestner, T. F. Serwold, L. Chen, and E. E. Moore. Chimeric human calcitonin and glucagon receptors reveal two dissociable calcitonin interaction sites. *Biochemistry* 34: 1050–1057, 1995.
  100. Suarez, F., J. J. Lebrun, D. Lecossier, B. Escoubet, C. Coureau, and C. Silve. Expression and modulation of the parathyroid hormone (PTH)/PTH-related peptide receptor messenger ribonucleic acid in skin fibroblasts from patients with type 1b pseudohypoparathyroidism. *J. Clin. Endocrinol. Metab.* 80: 965–970, 1995.
  101. Suva, L. J., G. A. Winslow, R. E. Wettenhall, R. G. Hammonds, J. M. Moseley, H. Diefenbach-Jagger, C. P. Rodda, B. E. Kemp, H. Rodriguez, E. Y. Chen, P. J. Hudson, J. T. Martin, and W. I. Wood. A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. *Science* 237: 893–896, 1987.
  102. Takasu, H., H. Baba, N. Inomata, Y. Uchiyama, N. Kubota, K. Kumaki, A. Matsumoto, K. Nakajima, T. Kimura, S. Sakakibara, T. Fujita, K. Chihara, and I. Nagai. The 69–84 amino acid region of the parathyroid hormone molecule is essential for the interaction of the hormone with the binding sites with carboxyl-terminal specificity. *Endocrinology* 137: 5537–5543, 1996.
  103. Takasu, H., and F. R. Bringhurst. Type-1 parathyroid hormone (PTH)/PTH-related peptide (PTHrP) receptors activate phospholipase C in response to carboxyl-truncated analogs of PTH-(1–34). *Endocrinology* 139: 4293–4299, 1998.
  104. Takasu, H., J. Guo, and F. Bringhurst. Dual signaling and ligand selectivity of the human PTH/PTHrP receptor. *J. Bone Miner. Res.* 14: 11–20, 1999.
  105. Tawfeek, H. A., J. Che, F. Qian, and A. Abou-Samra. Internalization of a green fluorescent protein-tagged rat PTH/PTHrP receptor (Abstract). *Bone* 23, Suppl. 1: S164, 1998.
  106. Tian, J., M. Smorgorzewski, L. Kedes, and S. G. Massry. Parathyroid hormone-parathyroid hormone related protein receptor messenger RNA is present in many tissues besides the kidney. *J. Am. Soc. Nephrol.* 13: 210–213, 1993.
  107. Tregear, G. W., J. Van Rietschoten, E. Greene, H. T. Keutmann, H. D. Niall, B. Reit, J. A. Parsons, and J. T. Potts, Jr. Bovine parathyroid hormone: minimum chain length of synthetic peptide required for biological activity. *Endocrinology* 93: 1349–1353, 1973.
  108. Turner, P. R., T. Bambino, and R. A. Nissenson. Mutations of neighboring polar residues on the second transmembrane helix disrupt signaling by the parathyroid hormone receptor. *Mol. Endocrinol.* 10: 132–139, 1996.
  109. Turner, P. R., T. Bambino, and R. A. Nissenson. A putative selectivity filter in the G-protein-coupled receptors for parathyroid hormone and secretin. *J. Biol. Chem.* 271: 9205–9208, 1996.
  110. Turner, P. R., S. Mefford, T. Bambino, and R. A. Nissenson. Transmembrane residues together with the amino-terminus limit the response of the parathyroid hormone (PTH) 2 receptor to PTH-related peptide. *J. Biol. Chem.* 273: 3830–3837, 1998.
  111. Urena, P., X. F. Kong, A. B. Abou-Samra, H. Jüppner, H. M. Kronenberg, J. T. Potts, Jr., and G. V. Segre. Parathyroid hormone (PTH)/PTH-related peptide (PTHrP) receptor mRNA are widely distributed in rat tissues. *Endocrinology* 133: 617–623, 1993.
  112. Usdin, T. B., T. I. Bonner, G. Harta, and E. Mezey. Distribution of PTH-2 receptor messenger RNA in rat. *Endocrinology* 137: 4285–4297, 1996.
  113. Usdin, T. B., C. Gruber, and T. I. Bonner. Identification and functional expression of a receptor selectively recognizing parathyroid hormone, the PTH2 receptor. *J. Biol. Chem.* 270: 15455–15458, 1995.
  114. van Dop, C. Pseudohypoparathyroidism: clinical and molecular aspects. *Semin. Nephrol.* 9: 168–178, 1989.
  115. Vasavada, R. C., C. Cavaliere, A. J. D'Ercole, P. Dann, W. J. Burtis, A. L. Madlener, K. Zawulich, W. M. Philbrick, and A. F. Stewart. Overexpression of parathyroid hormone-related protein in the pancreatic islet of transgenic mice causes islet hyperplasia, hyperinsulinemia and hypoglycemia. *J. Biol. Chem.* 271: 1200–1208, 1996.
  116. Vortkamp, A., K. Lee, B. Lanske, G. V. Segre, H. M. Kronenberg, and C. J. Tabin. Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. *Science* 273: 613–622, 1996.
  117. Weinstein, L. S. Albright hereditary osteodystrophy, pseudohypoparathyroidism, and G<sub>s</sub> deficiency. In: *G Proteins, Receptors, and Disease*, edited by A. M. Spiegel. Totowa, NJ: Humana, 1998, p. 23–56.
  118. Weir, E. C., W. M. Philbrick, M. Amling, L. A. Neff, R. Baron, and A. E. Broadus. Targeted overexpression of parathyroid hormone-related peptide in chondrocytes causes skel-

- etal dysplasia and delayed endochondral bone formation. *Proc. Natl. Acad. Sci. USA* 93: 10240–10245, 1996.
119. **Wu, T. L., R. C. Vasavada, K. Yang, T. Massfelder, M. Ganz, S. K. Abbas, A. D. Care, and A. F. Stewart.** Structural and physiological characterization of the mid-region secretory species of parathyroid hormone-related protein. *J. Biol. Chem.* 271: 24371–24381, 1996.
120. **Wysolmerski, J. J., A. E. Broadus, J. Zhou, E. Fuchs, L. M. Milstone, and W. M. Philbrick.** Overexpression of parathyroid hormone-related protein in the skin of transgenic mice interferes with hair follicle development. *Proc. Natl. Acad. Sci. USA* 91: 1133–1137, 1994.
121. **Wysolmerski, J. J., J. F. McCaughern-Carucci, A. G. Daifotis, A. E. Broadus, and W. M. Philbrick.** Overexpression of parathyroid hormone-related protein or parathyroid hormone in transgenic mice impairs branching morphogenesis during mammary gland development. *Development* 121: 3539–3547, 1996.
122. **Yasuda, H., N. Shima, N. Nakagawa, K. Yamaguchi, M. Kinosaki, S. I. Mochizuki, A. Tomoyasu, K. Yano, M. Goto, A. Murakami, E. Tsuda, T. Morinaga, K. Higashio, N. Udagawa, N. Takahashi, and T. Suda.** Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc. Natl. Acad. Sci. USA* 95: 3597–3602, 1998.
123. **Zhang, P., A. S. Jobert, A. Couvineau, and C. Silve.** A homozygous inactivating mutation in the parathyroid hormone/parathyroid hormone-related peptide receptor causing Blomstrand chondrodysplasia. *J. Clin. Endocrinol. Metab.* 83: 3373–3376, 1998.

